THE PATHOLOGICAL ANATOMY OF EXPERIMENTAL NAGANA.*

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INTRODUCTION.

ALTHOUGH various workers have described the gross pathological anatomy of trypanosomiasis, few have given any details of the microscopical anatomy of the condition.

In their report on nagana in 1898, Kanthack, Durham, and Blandford[1] state that the number of red blood cells are decreased in this condition; that the hemoglobin is decreased proportionately to the red blood cells; that normoblasts may be found in the circulating blood; and that leucocytosis is frequently present. They found that the lymph glands and spleen were enlarged in rats and mice, and that the liver showed fatty change. These changes were not so marked in the guinea pig, but the bone marrow was frequently hyperplastic, and the spleen, liver, and kidneys contained varying amounts of iron-containing blood pigment.

Plimmer and Bradford[2] (1899, 1901) added no new microscopical findings. In 1902, Laveran and Mesnil[3] published the results of their study on the morphology of *Trypanosoma Brucei* and found that the enlargement of the spleen was due to a congestion without appreciable microscopical changes.

In the same year Voges[4] published his work on mal de caderas, in which he found an increase of the fluids in all the body cavities. He also found that the lymph glands were frequently enlarged,

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[1] KANTHACK, DURHAM, AND BLANDFORD, *Proc. Roy. Soc.*, 1898, 64, pp. 110-18; translation by NUTTALL in *Hygienische Rundschau*, 1898, 8, p. 1185.

[2] PLIMMER AND BRADFORD, *Proc. Roy. Soc.*, 1899, 65, pp. 274-81; *Quart. Jour. Micr. Sci.*, 1901, 45, p. 449.

[3] LAVERAN AND MESNIL, *Ann. de l'Inst. Past.*, 1902, 16, pp. 1-55.

[4] VOGES, *Ztschr. f. Hyg.*, 1902, 39, pp. 323-72.

and that the spleen was always increased in size, due in many cases to hyperplasia of the trabeculæ. He stated that lungs and heart showed no change; that the liver and kidneys may be enlarged, the latter frequently showing a hemorrhagic nephritis.

In 1903, Elmassian and Migone[1] added another microscopical finding to trypanosomiasis, viz., congestion of the membranes of the brain and the frequent increase in the amount of the subarachnoid fluid.

In the same year Bruce and Nabarro[2] reported the autopsy findings of a number of cases of sleeping sickness. In general the cases show no signs of acute inflammation, but merely a flattening of the convolutions, an injection of vessels, and an excess of subarachnoid fluid.

A short time after this, Manson[3] reported that Mott and Low found the brain of an English woman dying from sleeping sickness to show a perivascular, small cell infiltration.

While the above is not the complete literature of the work, it contains, as far as is possible, all the findings reported by the various workers. In view of the above, and considering the fact that new points might be added to the pathology of experimental nagana, the following work was undertaken.

Some of these animals had been inoculated with pure cultures, others with one or two drops each of infected blood, and others with one or two cubic centimeters of a potassium citrate emulsion of the various organs of an infected animal. The difference of pathological picture presented by the three methods was only one of degree, not one of quality, that resulting from inoculation of pure cultures being least marked in degree.

GROSS PATHOLOGICAL ANATOMY.

General appearance.—Body emaciated. Sclera frequently yellowish. Hair coarse, dry, and rough.

Brain.—Meninges lax, moist; varying degree of congestion; not thickened. Subarachnoid fluid increased. Convolutions of brain slightly flattened; surface smooth, shiny, moist. Vessels of pia injected. Cut surface; moist; vessels congested. Fluid in ventricles slightly increased; choroid plexus injected.

[1] ELMASSIAN AND MIGONE, *Ann. de l'Inst. Past.*, 1903, 17, pp. 241-67.

[2] BRUCE AND NABARRO, *Reports of the Sleeping Sickness Commission*, No. 1, Aug., 1903 (London: Harrison & Sons); BRUCE, NABARRO, AND GREIG, *ibid.*, No. 4, Nov., 1903.

[3] MANSON, *Brit. Med. Jour.*, 1903, 2, p. 1462, Dec. 5.

Mediastinum.—Mediastinal fat small in amount. Thymus increased in size; surrounding fat pinkish in color. Frequently enlarged lymph glands present in mediastinum.

Heart and pericardium.—Pericardium thin, slightly distended; fluid straw color and slightly increased. Epicardium smooth, shiny, moist, not thickened. Myocardium distended with currant jelly clots; walls slightly thickened, otherwise negative. Endocardium clear, shiny, moist.

Lungs.—Vary in color from light pink to dark red in a given lung. As a whole the lung floats on water, but frequently parts of lung would sink. Such parts would be dark in color, airless, and give rich exudate on pressure from alveoli. Bronchi negative. Vessels congested.

Spleen.—Greatly enlarged. Edges rounded, and organ as a whole curved upon hilus. Surface smooth for most part, but frequently would have small fibrin tags attached. Peritoneum over spleen thickened. Organ firm; consistency increased. Cut surface: Follicles increased in size; stand out as white or grayish spots. Pulp reticulum and trabeculæ increased in size and amount. Pulp flow very slight.

Liver.—Slightly enlarged. Surface smooth, shiny, moist. Dark red in color. Consistency normal. Cut surface bleeds freely. Central vein distended. Central bone of liver lobule slightly yellow in color. Glisson's capsule not increased in rats or in mice, but slightly increased in guinea pigs.

Intestines.—Slightly distended. Serosa smooth, shiny, moist. Walls negative on section. Pancreas negative.

Kidneys.—Slightly increased in size, red in color. Consistency slightly increased. Fatty capsule thin. Fibrous capsule not thickened; strips easily. Cut surface of kidney moist. Glomeruli stand out as small red points. Labyrinth slightly enlarged; all vessels congested. Adrenals negative.

Lymph glands.—Retroperitoneal, enlarged, pinkish, moderately firm.

External genitals.—Slightly edematous.

Ovaries or testicles.—Negative, except for slight edema and congestion.

Bone marrow.—Sternum and femur hyperplastic. Fatty marrow of femur replaced by varying amounts of lymphoid marrow.

MICROSCOPICAL ANATOMY.

Spleen.—Certain changes were constant in every section from every animal examined, but the degree of this change varied greatly. This variation seemed to have no relation with either the mode of injection, the injected material, or the time elapsing between the injection and death.

The follicles are enlarged in every section. The enlargement of a greater number of these follicles is due largely to an increase in the number of a certain form of cell. These cells are large, round or oval, somewhat larger than a polymorphonuclear; they possess a coarsely granular protoplasm, surrounding a round or oval, deeply staining nucleus, which is excentrically situated and whose chromatin is slightly bunched. With Ehrlich's triacid mixture the protoplasm of some of these cells shows fine or coarse granules staining a reddish purple. With eosin and methylene blue combination the greater number of these cells are seen to contain coarse granules staining a deep blue. Other enlarged follicles show two distinct zones—an inner lightly

staining, and an outer deeply staining zone. The inner zone is composed of cells whose protoplasm stains faintly, and whose nuclei are centrally placed and stain lightly. The outer zone is composed of cells resembling those first described above. Between these two zones transition forms are sometimes seen—cells which are smaller than the central cells, their chromatin more bunched, and their protoplasm showing scattered granules. The other cells which are found in the enlarged follicles are about the size of a red blood cell, contain practically no protoplasm, and possess a round, deeply staining, compact nucleus.

What has been said about the fact that the changes in the follicles have no relation to the mode of infection, etc., is also true of the pulp. The pulp in every case is congested. Sometimes this congestion may be central; at others it is seen only at the periphery of the organ. In the older cases the stroma is increased in a varying degree. The cells vary also. Cells resembling those of the first type described above are increased in a varying amount everywhere throughout the pulp. Scattered among them are small and large lymphocytes, and a few nucleated red blood cells. In some sections the lymphocytes are bunched in masses of ten to thirty. In other sections the stroma was increased greatly, and the cells seem to be arranged in cords along definite trabeculæ of pulp stroma.

Varying amounts of iron-containing pigment were present in every section of spleen, and in the case of this pigment a definite relation seemed to exist between the amount present and the time elapsing between the injection of the infected material and the time of death.

In spleens from animals injected with artificially grown trypanosomata the amount of iron-containing pigment which was deposited increased up to the eighteenth day; the spleens of animals dying on the twenty-first day were less; those of the twenty-second, still less; those of the twenty-third, slightly increased over the twenty-second; those of the twenty-fourth and fifth are increased to about the same amount as those of eighteen days. In spleens of animals dying on the twenty-ninth day the amount of iron-containing pigment is almost normal, but the spleens of the animals dying on the thirty-sixth day contain as much as those dying on the eighteenth day.

The increase in the amount of iron-containing pigment in the spleen of animals injected with the organ suspensions is seen earlier. The first great increase is on the eleventh day; falls to practically normal on the twenty-seventh; rises on the twenty-sixth to a greater amount than on the eleventh, to fall to normal on the twenty-eighth, where it remains till the sixty-first day, when it rises slightly, and remains so until the one hundred and fifth day.

Spleens from animals injected with infected blood show the greatest amount of iron-containing pigment. On the fifteenth day the first increase is at its height; falls on the nineteenth to practically normal; is increased again on the thirtieth to the thirty-sixth; falls again on about the fortieth, to rise again on the ninety-second.

Lymph glands.—All lymph glands show a great increase in the number of lymphocytes, accompanied by a greater or less hyperplasia of the stroma. In some glands taken from an animal which had been injected with one drop of

infected blood, and which lived for ninety two days, a large number of mono nucleated white blood cells are present. Some of these cells contain coarse oxyphile, others fine oxyphile cells. In the sinuses of some glands large round or oval hyaline cells are present which contain remains of red blood cells or granules of iron-containing pigment. The stroma of these glands are markedly hyperplastic.

Lymph glands taken from animals which were inoculated with cultures show no changes. Those from animals injected with organ suspensions show similar changes to the ninety-two-day blood inoculation animal, but the changes are less in degree.

Thymus.—The increase in size of the thymus is due to a simple hyperplasia of the lymphoid elements and the stroma.

Liver.—The liver cells at all stages show a cloudy swelling, varying in degree from the earliest to a stage where the outline of the liver cell is very poorly made out, and the cords of cells are a granular mass with poorly staining nuclei. Frequently these areas contain large unmbers of adult trypanosomata.

Fatty degeneration accompanies this cloudy swelling, but seems to vary in degree according to the method of inoculation. In those animals which were injected with cultures a small amount of fatty degeneration is seen in the livers of animals dying on the fifteenth day; those dying on the eighteenth day show a slight increase over the fifteenth-day animal, but animals dying later show no increase. Some parenchymatous cells of the livers from animals injected with 1–2 c.c. of the citrate suspension of organs of infected animals, and which died on the tenth day, show a moderate number of large fat drops. The protoplasm of practically all other cells is composed of highly refractive droplets. The cells from livers of animals dying on the fifteenth, twenty-sixth, and seventy-first days show less fatty degeneration; those of the twenty-second and twenty-eighth show about the same amount of fatty degeneration as those dying on the tenth; while those dying on the one hundred and fifteenth day show practically no change.

Parenchymatous cells from livers of animals inoculated with one drop of infected blood show the greatest change. Cells from the liver of an animal dying on the ninth day show a moderate fatty degeneration; those dying on the thirteenth to fifteenth, marked; those on the nineteenth, thirty-seventh, and ninety-second, less. Aside from cloudy swelling and fatty degeneration, the livers show nothing pathological, except an occasional variable leucocytosis.

In those animals which were inoculated with one drop of infected blood, and which died on the fourteenth day, the livers show a relatively large number of capillary endothelial cells withholding iron-containing pigment, but the liver cells are free. The liver or endothelial cells of all other animals show no iron-containing pigment.

Lungs.—The pulmonary vessels of all lungs coming to autopsy are greatly distended. The alveolar capillaries are so dilated and filled with blood that it is frequently difficult to outline the individual cells. Between the blood cells varying numbers of adult trypanosomata are present. In some areas the alveoli contain a granular exude inclosing desquamated epithelial cells. In

other areas the alveolar spaces are compressed, and their walls are folded upon themselves. In other areas some alveoli contain red blood cells, leucocytes, a small amount of stringy or granular fibrin, and desquamated epithelium. Some bronchi show a marked mucous degeneration.

Heart, adrenals, salivary glands, pancreas, testis, ovary, are negative except for a varying amount of acute congestion.

From the foregoing pathological histology it will be seen that the changes produced by *Trypanosoma Brucei* are those of an intoxication. The spleen shows a congestion, accompanied by hyperplasia of the pulp reticulum in some areas, and by a varying degree of hyperplasia of cells corresponding in morphological characteristics to myelocytes. The greater number of cells of the normal spleen are lymphocytes and the follicles are well outlined, but in all the spleens taken from animals infected with *Trypanosoma Brucei* the great enlargement is due to an increase in the stroma of the pulp, accompanied by an enlargement of the follicles. This enlargement of the follicles is due to an increase in the number of forms of cells. As mentioned above, the central zone of the follicles, which is composed largely of endothelial cells, is enlarged. Outside this is another zone, consisting of mononuclear cells composed of a coarsely granular protoplasm surrounding a single, deeply staining nucleus. The protoplasm of these cells contains both fine and coarse oxyphile granules. In other words, these cells resemble morphologically the cells designated as myelocytes in man. Between these two forms of cells are transition forms which are present in the boundary between the two zones, so that it is possible to say in many sections that the myelocyte develops from an endothelial cell.

Accompanying this marked hyperplasia and new formation of myelocytes, few red blood cells are seen and scattering giant cells resembling those of the bone marrow. Many of these cells are seen to be developing from endothelial cells of the pulp. These cells have a single, lobulated, deeply staining nucleus, surrounded by a finely granular protoplasm.

Another important change in the spleen is the deposit of iron-containing pigment—hemosiderin. From the description it can be seen that the amount of iron-containing pigment in the

spleen increases until the fifteenth day; then the amount is less for eight to ten days, when there is another increase, followed by another decrease, which in turn is followed by another increase.

The explanation for this seems to be that the body energies are able by certain explosive efforts to overcome the agent which is causing the destruction of red blood cells, which is shown by a decrease in the amount of hemosiderin in the spleen. These energies are unable completely to destroy these agents, and the animal eventually succumbs.

What the destructive agent may be it is difficult to say. That the Trypanosomata themselves do not attack the cells is clear beyond doubt; consequently, it is quite probable that they excrete some substance which is hemolytic, and the end result of this process is the hemosiderin found in the spleen.

On the part of the other hemopoietic organs, the marrow of the long bones becomes hyperplastic, as do the lymph glands. Here it may be added that lymph glands taken from animals whose spleen shows the greatest hemosiderin contain a few hyaline cells with remains of red blood cells.

Plimmer and Bradford, and Laveran and Mesnil, have suggested that *Trypanosoma Brucei* produces its effects by means of some toxin which it forms, but these workers could not demonstrate the toxin. Nevertheless, from the histological findings given above, it seems very conclusive that the pathological changes are due to a toxin.

To sum up briefly the pathology of experimental nagana, it may be said that this disease is caused by *Trypanosoma Brucei*, which produces a mild intoxication acting chiefly on the blood and blood-forming organs.

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